

Role of Type I Secretion in Pierce's Disease

Project Leader:

Dean W. Gabriel
Plant Pathology Department
University of Florida
Gainesville, FL 32611

Postdoctoral Associate:

Joseph Reddy
Plant Pathology Department
University of Florida
Gainesville, FL 32611

Cooperator:

Donald Hopkins
Mid Florida Res. Edu. Center
University of Florida
Apopka, FL

Research Associate:

Stephanie Reddy
Plant Pathology Department
University of Florida
Gainesville, FL 32611

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Working hypothesis:



- Leaf scorch (PD) and necrotic leaf lesions (CVC) not typical of vascular blockage alone
- Type I effector secretion machine available for protein "toxin" secretion

Mechanism/s of the disease:

-Growth regulators

-Effectors ("Toxins")?

Multiple RTX effectors
found in *Temecula* genome:

Hemolysins:

PD0143

PD0282

PD0305

PD0536

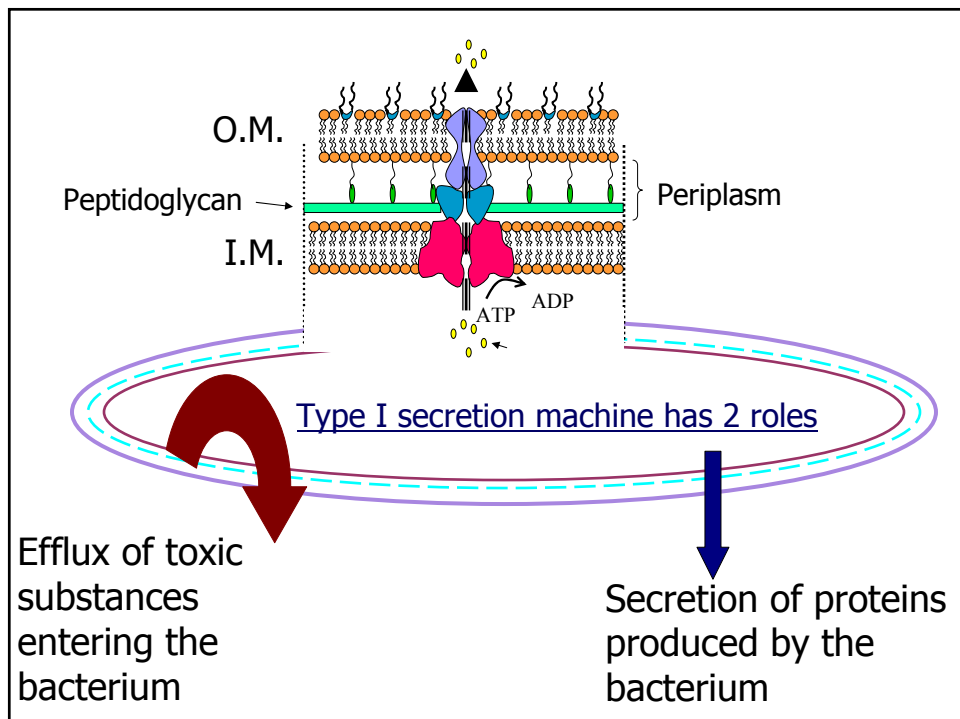
Colicin V precursors:

PD0852

PD0216

Bacteriocin:

PD1427



Type I secretion

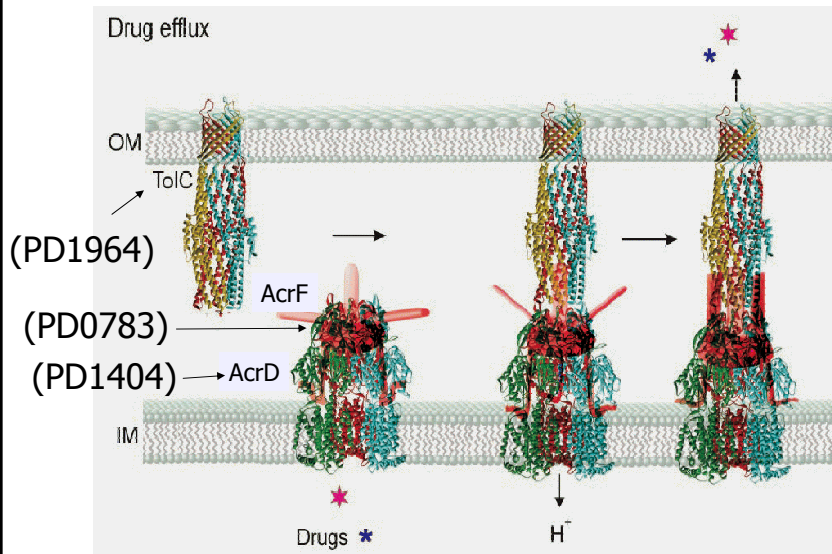


Figure from Koronakis et al. 2004 Ann. Rev. Biochem. 73:467

Type I secretion

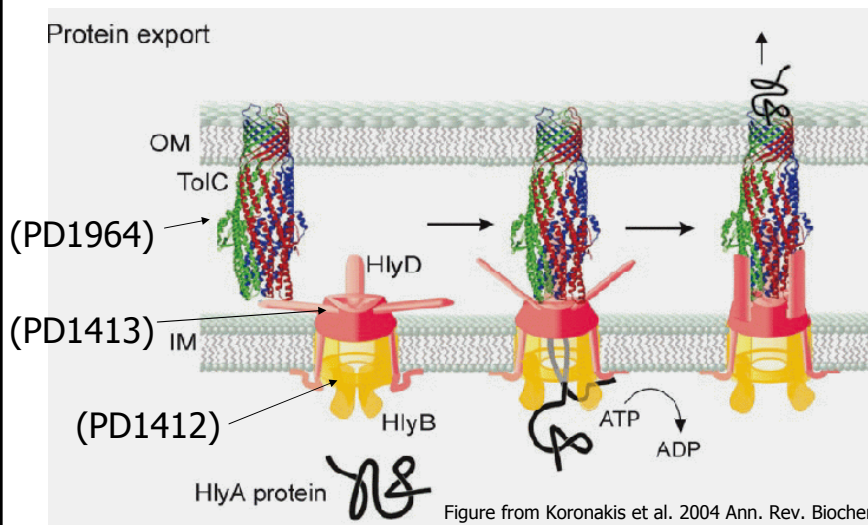
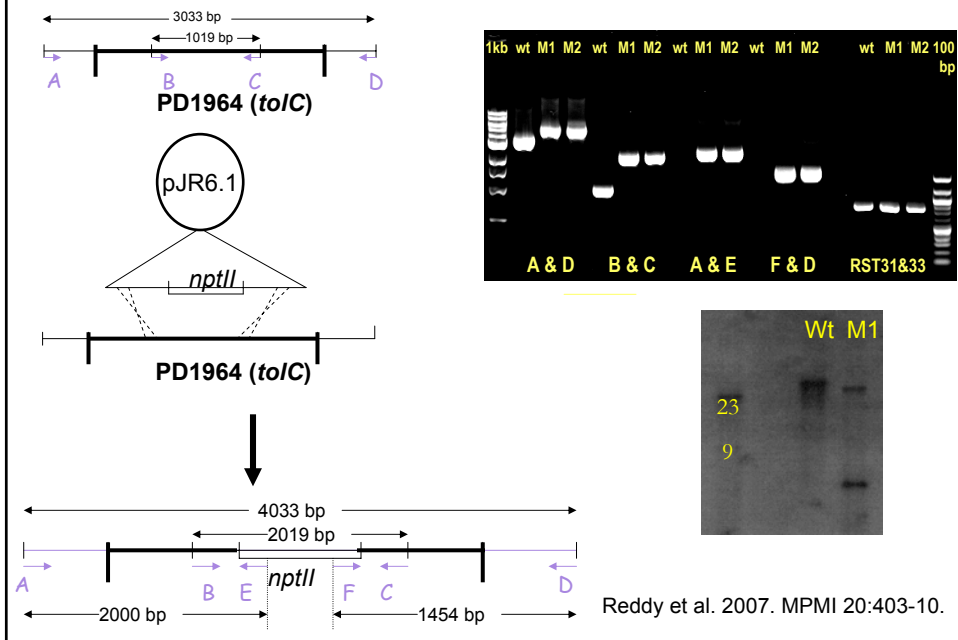


Figure from Koronakis et al. 2004 Ann. Rev. Biochem. 73:467

Two potential toxin secretion systems: HlyD (PD1413)/ HlyB (PD1412) & CvaA (PD0496)/ CvaB (PD0499)

Marker exchange of *toI/C* in Temecula



toI/C::nptII mutants are completely nonpathogenic...



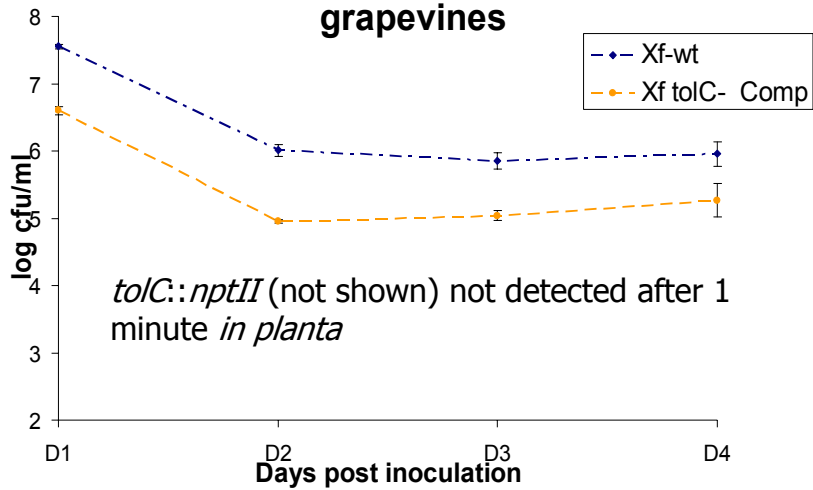
PD-T Wild Type

64 dpi

Type I secretion/efflux *toI/C*⁻

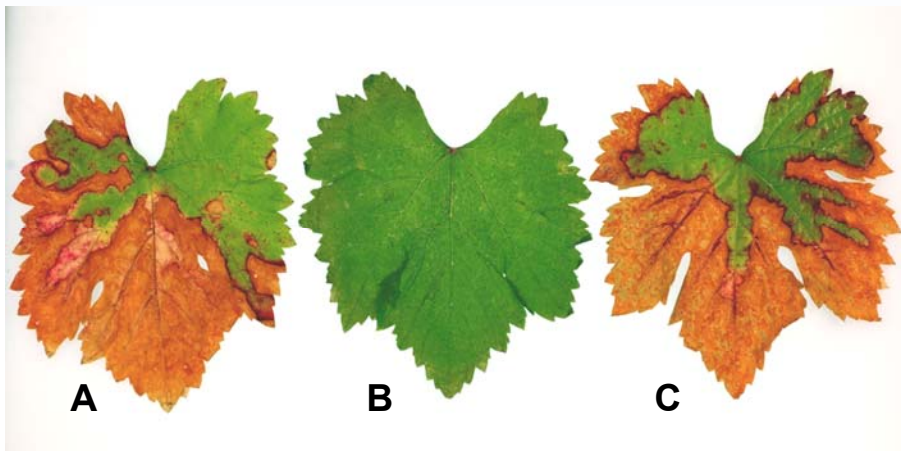
Reddy et al. 2007. MPMI 20:403-10.

...and cannot even be detected after
inoculation *in Vitis vinifera*
grapevines



Reddy et al. 2007. MPMI 20:403-10.

...and are complemented using pJR22.2 (*tolC*⁺)



Leaves at 60 days after inoculation. pJR22.2 has *Bordatella* rep.

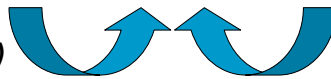
Reddy et al. 2007. MPMI 20:403-10.

...and knockouts of all other Type I system components exhibit reduced pathogenicity.

Xf Temecula (***tolC***)



Drug efflux
(*acrF* :: *nptII*)
(*acrD* :: *nptII*)



Protein Secretion
(*hlyBD* :: *nptII*)
(*cvaA* :: *nptII*)
(*cvaB* :: *nptII*)

var. Carignane



Type I secretion

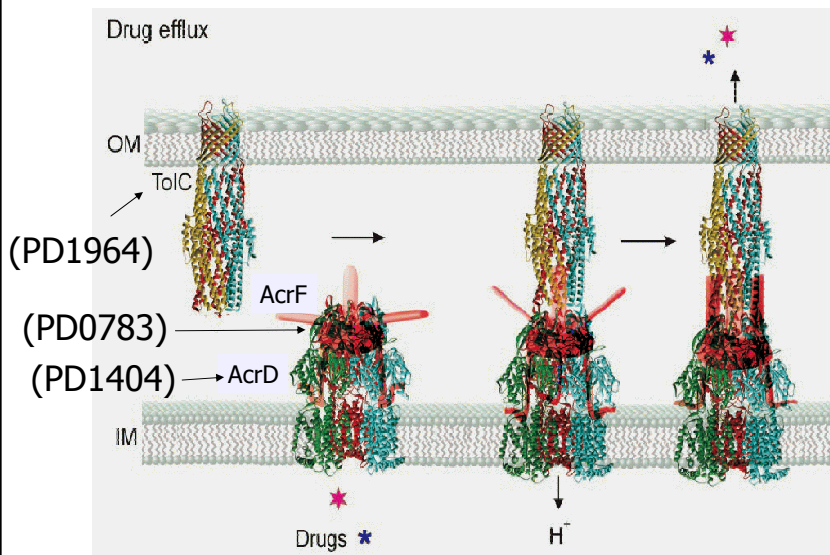


Figure from Koronakis et al. 2004 Ann. Rev. Biochem. 73:467



Xf is more sensitive to phytoalexins than *Erwinia*...

Chemical	MIC ($\mu\text{g/ml}$)			difference
	Temecula	<i>E. chrysanthemi</i> *	<i>E. amylovora</i> **	
Berberine	25	.02	.02	80X
Genistein	5	0.5	NT	200X
Rhein	50	.05	.05	20X

* Data from Barabote et al. (2003). **Data from Burse et al. (2004)

... and Xf MDR efflux mutants are 1000X more sensitive to phytoalexins than wt

Chemical	MIC ($\mu\text{g/ml}$)			difference
	Temecula	<i>tolC</i> ⁻	<i>acrF</i> ⁻	
Berberine	25	.02	.02	1000X
Genistein	5	0.5	NT	10X
Rhein	50	.05	.05	1000X

Reddy et al. 2007. MPMI 20:403-10.

...but not grape sap, even if not "running"...

unless the grape plant is first:

- wounded?
- frozen?
- elicited?
- sap is more concentrated?

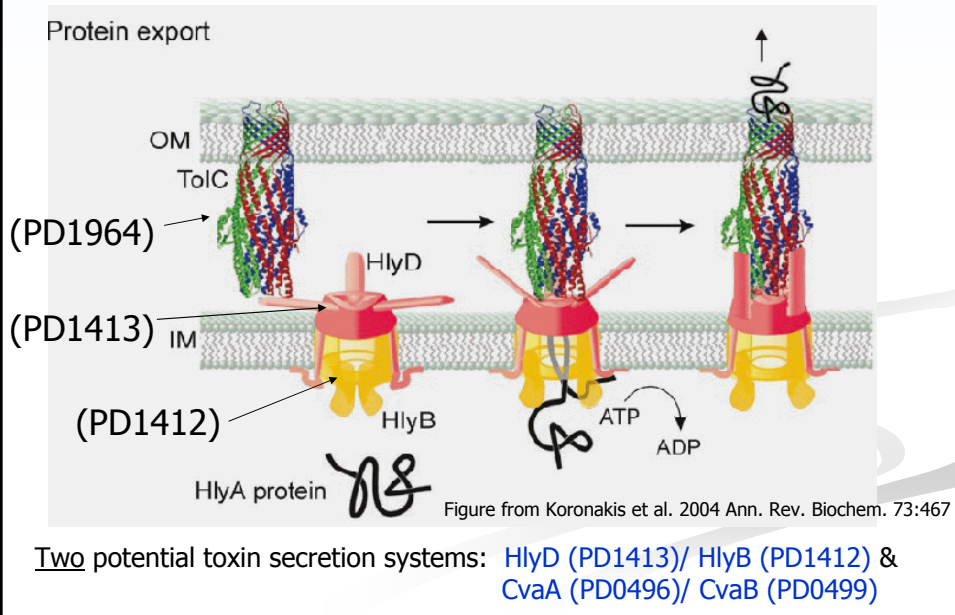
yet *tolC*⁻ never show disease...

why???

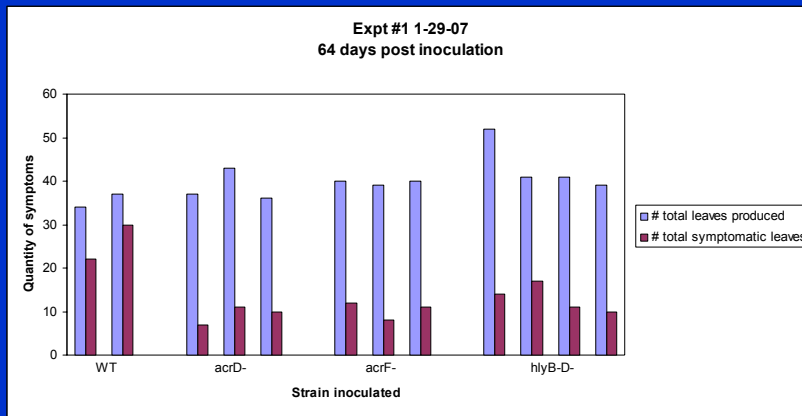
Answer may provide a control.



Type I secretion

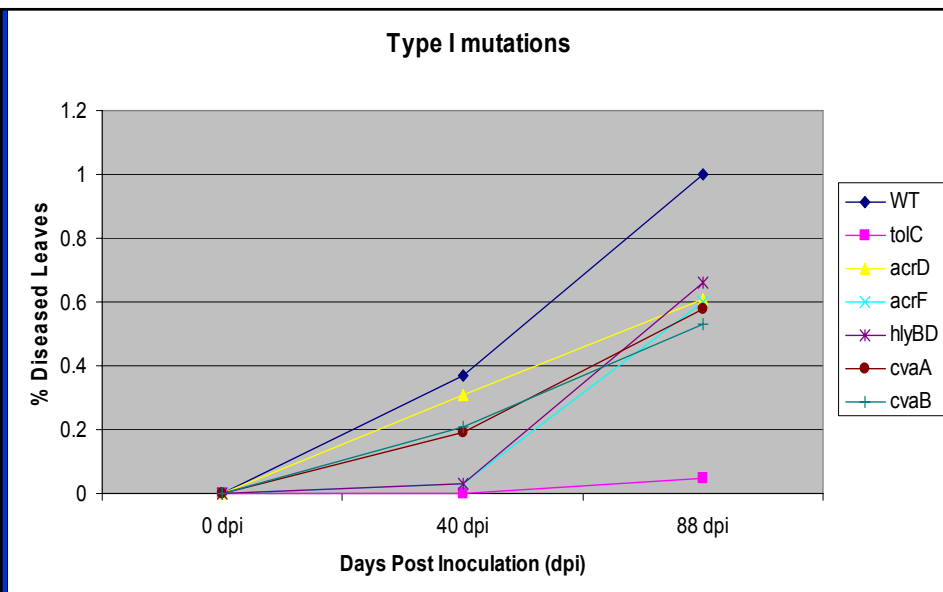






total leaves produced = # asymptomatic (healthy) leaves,
symptomatic (diseased) leaves, # bare petioles, # bare nodes

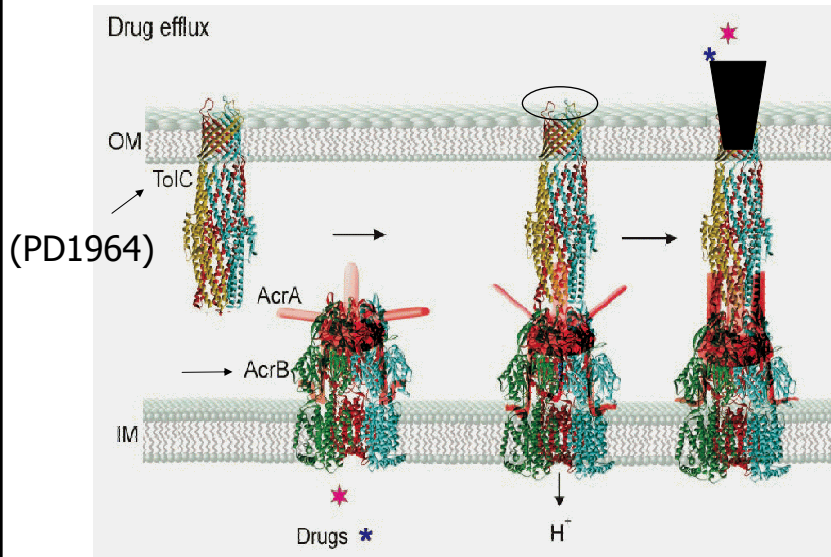
total symptomatic leaves = # symptomatic leaves, # bare petioles, # bare nodes



MDR efflux: *acrD*, *acrF*, *tolC*

Type I effector secretion: *hlyB*, *hlyD*, *cvaA*, *cvaB*, *tolC*

TolC is externally exposed and might be blocked by chemicals or engineered plants. To date, anti-TolC antibodies and aptamers fail to bind.



Conclusions

- pBBR1MCS-5 is sufficiently stable for Xf complementation
- *X. fastidiosa* absolutely requires *tolC* for disease and *in planta* survival; MDR efflux critical. **Not clear why sap has no detected effect**, although crushed tissue kills *tolC*⁻ quickly; therefore artificial elicitation is a potential strategy for PD control.
- Knockouts of all other (redundant) Xf Type I machine components exhibit reduced pathogenicity; protein "toxin" secretion likely important for PD.
- TolC has exposed region; therefore presents a potential target for drugs/antibody strategy to control this pathogen

Funding: U.C. Davis subaward from USDA Cooperative Agreement SA6477